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Inhibitory effects of glycolipids fraction from spinach on mammalian DNA polymerase activity and human cancer cell proliferation☆

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Abstract

We succeeded in purifying the fraction containing the major glycolipids in monogalactosyl diacylglycerol, digalactosyl diacylglycerol and sulfoquinovosyl diacylglycerol (SQDG) from dried vegetables. This glycolipids fraction was an inhibitor of DNA polymerase α (pol α) in vitro and also the proliferation of human cancer cells. In this study, eight common vegetables were investigated in terms of the glycolipids fraction, the amounts of major glycolipids, mammalian DNA polymerase inhibitory activity and antiproliferative activity toward human cancer cells. Green tea possessed the largest amount of glycolipids overall. Spinach contained the largest amount of SQDG, followed by parsley, green onion, chive, sweet pepper, green tea, carrot and garlic. Spinach had the strongest inhibitory effect on pol α activity and human cancer cell proliferation. A significant correlation was found between SQDG content and inhibition of DNA polymerase. Therefore, the inhibition of pol α activity by SQDG may lead to cell growth suppression. Of the six subspecies of spinach (*Spinacia oleracea*) tested, "Anna" had the largest amount of SQDG, strongest inhibitory activity toward DNA polymerase and greatest effect on human cancer cell proliferation. Based on these results, the glycolipids fraction from spinach is potentially a source of food material for a novel anticancer activity.

Keywords: Glycolipids; SQDG (sulfoquinovosyl diacylglycerol); Spinach; DNA polymerase α; Enzyme inhibitor; Anticancer agent

1. Introduction

In spite the many advances in cancer treatment, chemotherapy for solid tumors is still greatly limited by a lack of selective anticancer drugs and by the recurrence of drug-resistant tumors. Finding a source of novel chemotherapeutics continues to be a focus of effort. Diets rich in vegetables are known to reduce cancer risk, implicating edible plants as potential sources of anticancer agents.

Multiple organisms are known to contain at least 14 types of DNA polymerase [1]. DNA polymerases catalyze both DNA replication and repair [1,2]. DNA polymerase inhibitors could be employed as anticancer chemotherapy agents because they inhibit cell proliferation. Based on this idea, we have searched for and found many new DNA polymerase inhibitors over the past 9 years, for example, long-chain fatty acids and their derivatives [3–7], bile acids such as lithocholic acid [8,9], terpenoids [10–12], flavonoids [13,14], sulfate-

Abbreviations: DGDG, digalactosyl diacylglycerol; dTTP, 2′-deoxythymidine 5′-triphosphate; MGDG, monogalactosyl diacylglycerol; pol, DNA-directed DNA polymerase (EC 2.7.7.7); SQDG, sulfoquinovosyl diacylglycerol; TLC, thin-layer chromatography.

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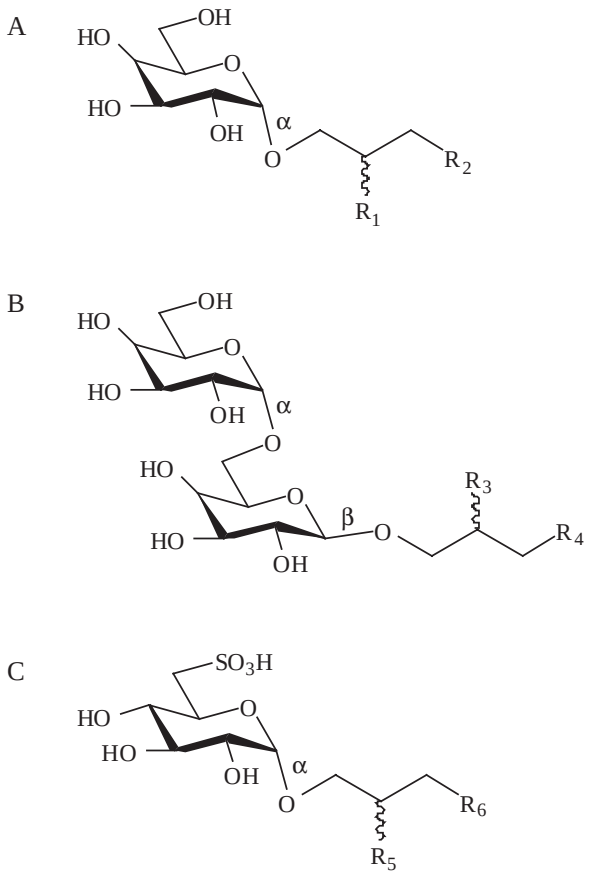

Fig. 1. Chemical structures of major glycolipids in the glycolipids fraction from vegetables. (A) MGDG. (B) DGDG. (C) SQDG. R_1 to R_6 in these structures are fatty acids.

containing glycolipids [15–23], vitamin A-related compounds [24], vitamin B_6 compounds [25], vitamin D_2 and D_3 [26], and nucleotide analogs [27,28] from natural resources.

Of these, sulfoglycolipids in the class sulfoquinovosyl diacylglycerol (SQDG, Fig. 1C) from a fern [15] and an alga [16,17] were particularly potent inhibitors and we concentrated on their anticancer effects in vivo. Sulfoquinovosyl diacylglycerol was not only potent inhibitors of the DNA polymerases in vitro [15–22], but also of human lung cancer in vivo [23]. This glycolipid shows promise as an agent for cancer chemotherapy.

Sulfoquinovosyl diacylglycerol is a major glycolipid of the chloroplast membrane in plants [29]. We have widely screened for a glycolipids fraction containing SQDG from common vegetables which show such inhibitory activities. The purpose of this report is to screen for vegetables with a glycolipids fraction that can inhibit DNA polymerase activities and human cancer cell proliferation.

2. Materials and methods

2.1. Materials

Nucleotides and chemically synthesized DNA template-primers such as [^{3}H]-2′-deoxythymidine 5′-triphosphate (dTTP, 43 Ci/mmol) and poly(dA), oligo(dT)$_{12–18}$ were purchased from Amersham Biosciences (Buckinghamshire, UK). Diaion HP-20 was obtained from Mitsubishi Chemical (Tokyo, Japan). Precoated Silica-Gel 60 plates (10×20 cm, 0.25-mm layer thickness) for thin-layer chromatography (TLC) were purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade and were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Sample preparation

Fresh vegetables [carrot (*Daucus carota*), chive (*Allium tuberosum*), garlic (*Allium sativum*), green onion (*Allium fistulosum*), green tea (*Thea sinensis*), parsley (*Petroselinum crispum*), spinach (*Spinacia oleracea*) and sweet pepper (*Capsicum annuum* var. *angulosum*)] were purchased from a local supermarket (Kobe-city, Hyogo-prefecture, Japan) in March 2004. The five subspecies of spinach (*S. oleracea*) (i.e., New Anna R4, Largo, T-881, Anna and Summer keep) were grown in Kohsei-machi, Kohka-gun, Shiga-prefecture, Japan. Samples were cleaned and dried at 40°C for 48 h before extraction.

2.3. Extraction and purification of the glycolipids fraction from vegetables

The purification methods for the glycolipids fraction from vegetables are shown in Fig. 2. The water-soluble substances were extracted from dried vegetables (2 g) with 100 ml of warm water (60°C). The tissue cake was added to 100 ml of warm ethanol (60°C), and the substances containing glycolipids were extracted. The 100% ethanol extract was diluted with water to be a 70% ethanol solution. The solution was subjected to Diaion HP-20 column chromatography, a

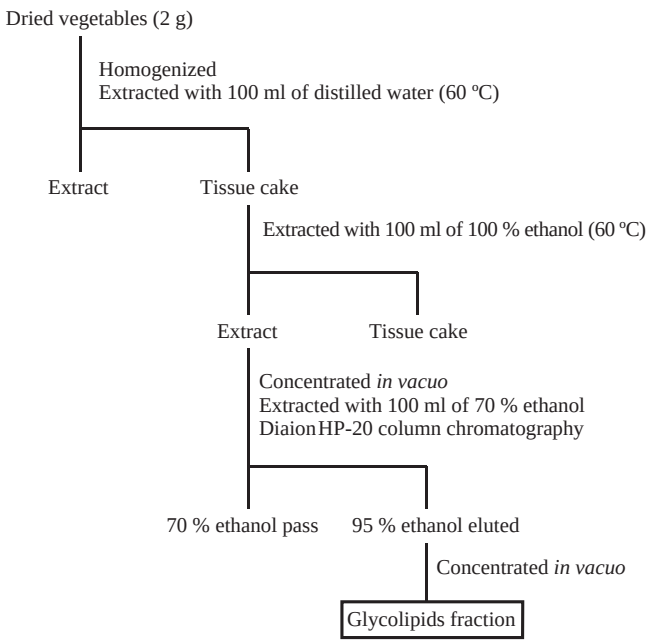

Fig. 2. The method of purifying the glycolipids fraction from dried vegetables.